REMARKS

Status of the Claims

Claims 1-4, 8-20, 39-40, 42, and 44 were previously cancelled. Claims 41 and 47 are cancelled herein.

Claims 21-23 and 30-38 stand withdrawn pursuant to the restriction requirement.

It is respectfully requested that the Examiner reconsider the withdrawal of claim 31. Claims 30-38 were withdrawn as being directed to a method rather than to the antibody fragments. Claim 31, however, is directed to the fragments, not the method. Claim 31, which previously depended from claims 1 and 24, is amended herein to depend only from claim 24, currently under consideration. It is respectfully submitted that it is proper to consider the patentability of claim 31 at this time

Applicant expressly preserves the right to pursue any of the withdrawn and cancelled claims in a subsequent continuation or divisional application.

The allowance of claims 5-7 is noted with appreciation.

Claims 24, 25, 45, and 46 are amended herein. Claims 26-29 and 43 were previously presented. New claims 48-56 are added herein.

The withdrawal of various previous rejections and objections as noted in pages 2-3 of the Office Action is noted with appreciation.

Claim rejections – 35 U.S.C. § 112, second paragraph

Independent claim 24 and claims dependent thereon stand rejected under 35 U.S.C. § 112, second paragraph with respect to the recitation of the term "derivative thereof" in reference to PEG. Although the term is defined in the specification at page 8, lines 4-9, the Examiner finds this definition indefinite in that it includes the words "for example" and "and the like." Without acquiescing in this ground of rejection, but solely to expedite prosecution of this application, the term has been deleted. Accordingly, it is respectfully submitted that the claim is no longer indefinite, and it is requested that the rejection be withdrawn.

Claim rejections – 35 U.S.C. § 103

The present invention relates, *inter alia*, to antibody Fab and Fab' fragments wherein the heavy chain and light chain are not covalently linked, the fragments having two or more effector molecules attached thereto, and wherein at least one of the effector molecules is attached to a cysteine in the light chain or heavy chain constant region (specification, p. 3, lines 1-14). The claimed invention is based on the surprising discovery that Fab and Fab' fragments having no covalent bond between the light and heavy chains, and having two or more large polymer effector molecules, at least one of which is attached to either the heavy or light chain, can exhibit antigen affinity comparable to wild-type antibody fragments (specification at page 3, lines 5-7).

Independent claim 24 has been amended to recite the embodiment wherein the heavy chain in the fragment is not covalently bonded to the light chain, an effector molecule is attached to an interchain cysteine of C_{L_α} and an interchain cysteine of C_H1 has been replaced by another amino acid. New claim 48 has been added to recite the embodiment wherein the heavy chain in the fragment is not covalently bonded to the light chain, an effector molecule is attached to an interchain cysteine of C_H1, and an interchain cysteine of C_L has been replaced by another amino acid. New claims 49-54 depend directly or indirectly from claim 48, and correspond to claims 27-29, 43, and 45-46, each of which depends directly or indirectly from claim 24. New independent claims 55 and 56 are similar to claims 24 and 48 respectively, and further recite the presence of the hinge and that any additional effector molecules are attached to the hinge

Claims 24-29, 41, 43, and 45-47 stand rejected under 35 U.S.C. 103 as obvious over Singh et al. as the primary reference in view of Hsei et al. and Humphreys. The rejection is respectfully traversed because the Office Action fails to take into consideration certain limitations of independent claim 24, both as previously presented and as currently amended, which limitations render claim 24 and the claims dependent thereon non-obvious over the art of record. As will be seen, new claims 48-56 are non-obvious for the same reasons.

The combination of the Singh, Hsei, and Humphreys references does not teach or suggest the present invention. The disclosures of each of these references will be discussed first singly, then in combination.

(a) The Singh reference

Singh et al. teaches a method of labeling *whole* antibodies, not fragments, with labels such as biotin-PEO-maleimide complex having a formula weight of 525.6 (p. 149, left column, lines 5-9). To accomplish the labeling, Singh teaches non-selective selenol-catalyzed reduction. The catalyzed reduction resulted in the addition of seven labels to the antibody in less than 5 minutes (p. 152, left column, first paragraph under "Results").

The labeled whole antibodies of the Singh disclosure are different from the conjugated antibody fragments as presently claimed in three important respects:

- Singh et al. uses whole antibodies, not antibody fragments.
- Singh et al. uses effector molecules smaller by at least an order of magnitude than those recited in claims 24 and 48.
- Singh et al. does not teach or suggest that an interchain cysteine in either C_H1 or C_L should be replaced by another amino acid. In fact, Fig. 1 of Singh et al. specifically shows the presence in the conjugated antibody of the S atoms from the original interchain cysteines, indicating that these cysteines have not been replaced.

The teachings of Singh relating to whole antibodies having their interchain cysteines intact and conjugated to small effector molecules would not teach or suggest to one of ordinary skill in the art of conjugated antibodies and antibody fragments that similar results could be achieved with conjugated antibody fragments having no constant region beyond the hinge, much larger effector molecules, and with the interchain cysteine in C_H1 or C_L being replaced by another amino acid. For the same reasons, the applicants respectfully submit that one of ordinary skill in the art would not combine the teachings of Singh (whole antibodies) with art relating to modifications of antibody fragments.

(b) The Hsei reference

Hsei et al. does not teach Fab or Fab' fragments having two or more PEG effector molecules and wherein either the cysteine of C_H1 has been replaced by another amino acid and the cysteine of the C_L is PEGylated, or the cysteine of C_L has been replaced by another amino acid and the cysteine of the C_H1 is PEGylated. On the contrary, when the fragment is Fab or Fab', and a polymer molecule is conjugated to a cysteine residue in the light or heavy chain, Hsei is very careful to say that the conjugate can have *only one polymer molecule* attached. In particular, Hsei et al. teaches the following (emphasis added):

- *F(ab')$_2$* fragments having no more than about two polymer molecules, wherein every polymer molecule is attached to a cysteine residue in the light or heavy chain that would ordinarily form a disulfide bridge linking the light and heavy chains (p. 23, lines 4-8; p. 24, line 24 – p. 25, line 32; p. 33, line 33 – p. 35, line 6).
- Fab, Fab', and Fab'-SH fragments having *no more than one* polymer molecule, wherein the polymer molecule is coupled to a cysteine residue in the light or *heavy* chain that would ordinarily form a disulfide bridge linking the light and heavy chains (p. 23, lines 9-14; p. 25, line 33 – p. 27, line 7; p. 35, line 7 – p. 36, line 21).
- Fab, Fab', and Fab'-SH fragment conjugates containing more than one polymer molecule, wherein *every* polymer molecule in the conjugate is *attached to the hinge region* (p. 23, line 15 – p. 24, line 23; page 31, line 25 – p, 33, line 32).
- Fab, Fab', and Fab'-SH fragment conjugates containing *no more than one* polymer molecule, wherein the polymer molecule in the conjugate is *attached to the hinge region* (p. 27, line 8 – p. 28, line 4).
- Fab, Fab', and Fab'-SH fragment conjugates containing *no more than one* polymer molecule (p. 36, line 22 – p. 37, line 31).

At page 28, line 32 – page 30, line 5, Hsei generally discusses conjugates of antibody fragments with more than one PEG molecule, but does not discuss the type of fragments, or where on the fragments the PEG molecules are attached. At page 30, line 6

– page 31, line 24, Hsei discusses Fab, Fab', Fab'-SH and $F(ab')_2$ fragments with more than one PEG molecule, but does not discuss where the PEG molecules are attached.

The Examiner's statement at page 10, lines 20-25 of the Office Action characterizing the teachings of Hsei is specifically traversed, as (1) p.23, line 15 – p. 24 line 23 is limited to embodiments wherein all the polymer molecules are attached to the *hinge* region; (2) p. 28, line 5 – p. 30 line 5 recites only the number and size of PEG molecules, but not the *type* of antibody fragment or *where* on the antibody fragment the PEG molecules are attached; and (3) p. 30, lines 6-36 recites fragments, and number and size of PEG molecules, but does not disclose *where* the PEG molecules are attached to the antibody fragments. Nor is Hsei enabling for Fab or Fab' fragments containing more than one effector molecule wherein the effector molecules are attached at any location other than the hinge.

The only Fab and Fab' fragments disclosed in Hsei that have more than one polymer molecule attached have *all* of the molecules attached *only* in the *hinge* region. Reading Hsei as a whole, one skilled in the art could not help but conclude that a Fab or Fab' antibody fragment conjugate with more than one polymer molecule attached and at least one molecule attached to a cysteine in the heavy or light chain (which cysteine would otherwise form the interchain bridge) would be at the very least undesirable, or more likely, impossible. Thus the Hsei reference actually teaches away from the surprising result obtained by the present invention.

(c) <u>The Humphreys reference</u>

Humphreys WO99/15549 is concerned with the production of dimeric $F(ab')_2$ fragments and is directed to a peptide TCPPCPXYCPPCPA, which, when part of a Fab' fragment, efficiently generates $F(ab')_2$ dimers (p. 2, lines 3-6). The peptide of interest contains four cysteines and is disposed in the Fab' *hinge* region; the dimers include two monomeric chains covalently linked through one, two, three, or four of the cysteine residues of the hinge region peptide of each chain (p. 6, lines 27-32). Effector molecules such as PEG can be attached to the dimers, preferably to the cysteine residues in the

peptide sequence (p. 8, line 33 – p. 9, line 36), which, as noted, are in the *hinge* region. In Example 1, the reference teaches the production of di-Fab' from Fab' constructs. Before the dimers are formed, the interchain disulphide bonds are removed from the Fab' constructs, and the interchain cysteines are changed to serines (p. 12, lines 23-30). The breaking of bonds and removal of the cysteines was done to minimize any possible incorrect interchain disulphide bonds between hinge regions and any other cysteines (p. 12, lines 25-26). No effector molecules were attached to these Fab' constructs (p. 12, line 23 – p. 19, line 4). The reference states that the presence of extra *hinge* cysteines above the single one found in the original Fab' was the most important factor in promoting di-Fab' formation in vivo (p. 18, lines 10-12), thus emphasizing the importance of the hinge region, not the interchain regions.

Example 2 is directed in part to *hinge specific* pegylation of $F(ab')_2$ molecules with modified hinges. $F(ab')_2$ is the only fragment type that is disclosed as being PEGylated (p. 19, line 5 – p.24, line 24). The only disulphide bonds of interest are in the hinge regions (p.22, line 32 – p. 23, line 5; Figure 4). PEGylation is discussed at p. 24, lines 8-24. Conditions were selected to retain the molecule as a dimeric species (p. 24, line 11). An assay showed 1.034 ± 0.090 thiols per $F(ab')_2$ being liberated for reaction (p. 24, lines 20-21), and the efficiency of PEGylation was $\leq 1.3\%$ (p. 24, lines 22-24). Thus, the Humphreys reference differs from the present invention in the following ways: i) Humphreys is directed to processes for making di-Fab' molecules, ii) Humphreys only PEGylates di-Fab' molecules, iii) only one PEG is attached per molecule, iv) the PEG is attached only in the hinge region, and v) the efficiency of attachment is $\leq 1.3\%$. Humphreys makes no mention of Fab or Fab' fragments having effector molecules attached, and no mention of any type of fragment having an effector molecule attached to an interchain cysteine. Thus, the Humphreys reference teaches away from the surprising result obtained in the present invention.

(d) <u>The combination of references does not render the presently claimed invention obvious</u>

The Examiner states that "It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced anti-

IL-8 Fab, Fab', Fab-SH and *F(ab')$_2$* fragments comprising a cysteine modified *hinge* region of SEQ ID Nos.:1-3 and PEGylated according to the selenol-catalyzed reduction of disulfides as taught by Singh et al., as well as pharmaceutical compositions...." (Office Action, page 6, lines 22 – 26, emphasis added). This is not a correct statement of the invention as presently claimed in claims 24 and 48 because (1) the present invention specifically excludes *F(ab')$_2$* fragments, (2) the claimed fragment has *more than one* effector molecule conjugated to it, (3) at least one of the effector molecules in the claimed fragment is bound to a cysteine *on the light chain or the heavy chain*, and (4) the interchain cysteine of the opposite chain of the claimed fragment has been replaced by another amino acid. Whether one skilled in the art would have had a reasonable expectation of success to produce the structure described by the Examiner is irrelevant to the obviousness of claims 24 and 48, because the structure described by the Examiner does not include the limitations of claim 24 and 48.

Furthermore, the data presented in the specification demonstrate the unexpected results achieved with the inventive antibody fragments. The Examiner is directed to Table 1 and antibodies pDPH225 and pDPH252, which show good levels of purification even at the elevated temperature of 60°C. See also, page 22, lines 2-7. Table 2 also shows that antibody pDPH225 has good Kd values of 8.5. The Examiner is respectfully reminded that a Kd value is a reciprocal value and thus the lower the lower the value the better. It is unexpected that these antibodies would exhibit such good stability under these conditions.

Addressing the Examiner's characterizations of the prior art at page 7 of the Office Action, while it is true that Singh teaches rapid reduction of interchain disulfides that are then labeled, the teachings of Singh are limited to reduction of *whole* antibodies, not fragments, and labeling with *small* effector molecules, not the *large* molecules recited in claims 24 and 48, nor does Singh et al. teach or suggest that the interchain cysteine of the opposite chain has been replaced by another amino acid. Furthermore, at page 7, lines 11-19, the Examiner discusses the teachings of Hsei and Humphreys relative to cysteines that may be engineered into the *hinge* region, but this suggests nothing about the attachment of effector molecules at the cysteines in *the light chain or heavy chain,* as recited in the present claims.

One of ordinary skill in the art would not be motivated to combine Singh with either Hsei or Humphreys, because one of ordinary skill in the art would not think that the teachings of Singh relating to whole antibodies and small labels are applicable to the teachings of Hsei and Humphreys relating to antibody fragments and large effector molecules, nor would such a combination result in the presently claimed antibody fragments.

The Examiner concludes at page 7, lines 19-28, "Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to produce anti-IL-8 Fab, Fab', Fab-SH and $F(ab')_2$ fragments comprising the cysteine containing hinge peptides of SEQ ID Nos:1-3 as taught by Humphreys and reduced using the selenol-catalyzed reduction of interchain disulfides to expose reactive thiols to which PEG molecules are attached...." To the contrary, there is no reason why one of ordinary skill in the art would have been motivated to combine these references, especially when one considers that whole antibodies and $F(ab')_2$ fragments are *not part of the presently claimed invention*. Singh et al. teaches small effector molecules on whole antibodies, not fragments; Hsei et al. teaches that Fab' fragments can have more than one effector molecule only when the effector molecules are all at the hinge; and Humphreys teaches effector molecules only on $F(ab')_2$ fragments, and then only in the hinge.

It is acknowledged that Singh et al. teaches a method for the rapid reduction of disulfides in an antibody. But that is not the issue. The issue is whether it would have been obvious to make a disulfide-reduced *fragment*, and then conjugate *large* effector molecules to the *cysteines in the light chain,* and then replace the C_H1 cysteine with another amino acid, as recited in claims 24 and 55, and to conjugate *large* effector molecules to the *cysteines in the heavy chain,* and then replace the C_L cysteine with another amino acid, as recited in claims 48 and 56, to produce a product that would have good stability and comparable affinity to antigen as wild type fragments. Hsei's and Humphreys' teachings about *hinge* region modifications, as discussed at pages 6-7 of the Office Action, do not relate either to conjugation of large effector molecules at the *light chain* and modification of the heavy chain constant region, or to conjugation of large effector molecules at the *heavy chain* constant region and modification of the light chain. In fact, the entire discussion at pages 6-7 of the Office Action does not address the

limitations of claim 24 as presented in the last amendment, or claims 48, 55, and 56 as presented herein.

The present rejection is based on selection from the prior of the specified elements from among numerous possible choices and combinations without guidance from the art to combine the elements as the applicants have done. This is in direct violation of Federal Circuit case law, which makes clear that an invention is not obvious when all that was obvious was to try to "vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art merely identifies certain parameters among numerous choices and varies them "where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible choices is likely to be successful." *Bayer Schering Pharma AG v. Barr Laboratories Inc.*, 91 USPQ2d 1569, 1572-73 (Fed. Cir. 2009).

Furthermore, the Examiner is applying a prohibited hindsight analysis, starting with the applicants' invention and using it as a template from which to extract unrelated teachings of the prior art. Yet the prior art, when taken as a whole, actually teaches away from the present invention. Singh only teaches whole antibodies as assay reactants, which would lead one away from the use of fragments. Hsei teaches that more than one effector molecule can be bound to a heavy or light chain only when the fragment is *F(ab')2*; all other fragments must have either (a) only one effector molecule attached to a heavy or light chain cysteine, or (b) more than one effector molecule, all of which are attached at the hinge region. This would lead one away from attempting to create an antibody Fab or Fab' fragment with more than one large effector molecule, at least one of which is attached to the heavy or light chain.

Thus combining Singh, Hsei, and Humphreys would not result in the structure of claims 24 and 48 as currently presented, namely a Fab or Fab' fragment having (a) two or more PEG effector molecules, one being attached at C_L or C_H1, or (b) the opposite respective interchain cysteine of C_H1 or C_L replaced by another amino acid, and there is no teaching or suggestion in the combined art to create such a structure.

As the Examiner correctly notes, the test of obviousness is what the combined teaching of these references would have suggested to those of ordinary skill in the art at the time the invention was made. The preparation of the claimed multi-PEGylated

fragments was difficult, as illustrated by Figure 1 of the present application, showing that success was not found for all reductants; instead, the inventors had to carefully determine which reductants would give them the desired multi-PEGylation. To the extent that the Examiner notes that some degree of predictability is required for obviousness, the fact that the applicants had to try several reductants before finding one that would work shows that this is an unpredictable art, further supporting the non-obviousness of the presently claimed invention.

In view of the foregoing, it is respectfully requested that the rejection under 35 U.S.C. 103 be withdrawn.

Double patenting

The double patenting rejection based on claims 7 and 10 of U.S 6,642,356 is respectfully traversed. The '356 patent is the U.S. equivalent of the Humphreys reference discussed above. As stated in the '356 Abstract, the reference discloses a peptide sequence that can be used as hinge regions in proteins, where they can be covalently coupled to achieve dimeric structures, for example, as found in antibodies. Independent claim 5 of the '356 patent recites an antibody fragment comprising one polypeptide chain having the recited amino acid sequence; claim 7 dependent on claim 5 recites that the fragment is a Fab or Fab' fragment, and claim 10 dependent on claim 7 recites the fragment with one or more effector or reporter molecules attached to it. As noted above the cited references do not make up for any of the noted deficiencies.

The '356 patent teaches nothing about binding an effector molecule to a cysteine on a light chain, and is solely directed to the use of particular peptides in the hinge region to create dimers. Thus one skilled in the art seeking to create Fab or Fab' fragments would not have looked to Humphreys '356. The present claims are not obvious variants of claims 7 and 10 of '356.

All of the Examiner's arguments are based on the modification of the hinge region with the specific sequence. In the present application, that feature is the subject only of dependent claim 28, from which no other claims depend. The present invention simply is not based on a particular hinge peptide. Instead, it is based on applicants' surprising discovery that an Fab or Fab' fragment could be produced having affinity for

antigen comparable to wild type antibody, yet have no disulfide bridge between the heavy and light chains, and have two or more large polymer molecules attached, at least one of which is attached to the light chain or the heavy chain constant region, and having the opposite C_H1 cysteine or light chain cysteine replaced by another amino acid.

The reference at page 15 of the Office Action to 37 CFR 1.78(c) is respectfully not understood, as neither the present application nor the '356 patent arose from a U.S. provisional application. Nevertheless, in order to resolve this issue, a statement confirming that the subject matters of the both present application and the '356 patent were commonly owned at the time the invention of this application was made will be submitted in due course, should the examiner maintain this ground of rejection.

CONCLUSION

As all points of rejection have been overcome, a Notice of Allowance is respectfully requested. The Examiner is invited to contact the applicant's undersigned representative if it is believed that a conference might further the prosecution of this matter.

Respectfully submitted,

Date: November 13, 2009

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